PCT/GB 99/01989

09/720358

#5

INVESTOR IN PEOPLE

Priority

5 K.Jones 5/22/01

The Patent Office

GB 99/01989

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

REC'D 30 JUL 1999
WIPO PCT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed G D Court.

Dated 23 July 1999

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

25 JUN 1998

Patents Form 1/77

s Act 1977.
16)

The Patent Office

1/77

25JUN98 E370726-4 D00354
P01/7700 25.00 - 9813613.8

Request for grant of a patent

(see the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

THE PATENT OFFICE
D
25 JUN 1998
RECEIVED BY POST

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1.	Your reference	PJA/G088175PGB
2.	Patent application number *(The Patent Office will fill in this part)*	**9813613.8**
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	THE VICTORIA UNIVERSITY OF MANCHESTER OXFORD ROAD MANCHESTER M13 9PL
	Patents ADP number *(if you know it)*	77394S001
	If the applicant is a corporate body, give the country/state of its incorporation	UNITED KINGDOM
4.	Title of the invention	PULSE RESPONSE ASSESSMENT
5.	Name of your agent *(if you have one)*	Marks & Clerk
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Sussex House 83-85 Mosley Street Manchester M2 3LG
	Patents ADP number *(if you know it)*	18004

		Country	Priority application number *(if you know it)*	Date of filing *(day/month/year)*
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

		Number of earlier application	Date of filing *(day/month/year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		

8.	Is a statement of Inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	6
Claim*(s)*	2
Abstract	1
Drawing*(s)*	1 a\

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of Inventorship and right to grant of a patent *(Patents Form 7/77*	
Request for preliminary examination and search *(Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(Please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature.. Date 24/06/98

MARKS & CLERK

12. Name and daytime telephone number of person to contact in the United Kingdom

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*
b) *Write your answers in capital letters using black ink or you may type them.*
c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*
d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*
e) *Once you have filled in the form you must remember to sign and date it.*
f) *For details of the fee and ways to pay please contact the Patent Office.*

PULSE RESPONSE ASSESSMENT

The present invention relates to a method and apparatus for assessing the characteristic response of a medium to an excitation pulse the duration of which causes the medium to emit a series of signals over a period of time which is long relative to the duration of the excitation pulse.

The present invention is particularly applicable to the assessment of the fluorescent decay but could be applied to any circumstances in which an excitation pulse triggers a response that has decay or other time dependent characteristics which continue for a substantial period of time after termination of the excitation pulse. The nature of the excitation pulse may be the same as or different from the nature of the emission, for example light trigger and light emission or chemical trigger and light emission, and the excited species need not be excited directly, for example excimer and fluorescence energy transfer where the excited species is not the emitting species.

In conventional fluorescence decay analysis, in which a series of photons are emitted after pulse excitation, the time interval between the excitation pulse and the first emitted photon is monitored. The sample is repeatedly excited to enable the accumulation of data representing the distribution of the arrival times of the first photons resulting from each excitation. A significant delay is required between successive excitations to ensure that aliasing does not occur, that is to ensure that a photon emission resulting from one excitation is not detected as the first emitted photon resulted from the next excitation. Typically the delay required is from 10 to 100 times the fluorescent lifetime. These delays, coupled with the need for repeated excitation, prevent high speed measurements being obtained.

It is an object of the present invention to obviate or mitigate the problems outlined above.

According to the present invention, there is provided a method for assessing the characteristic response of a medium to an excitation pulse of predetermined duration which causes the medium to emit a series of signals over a period of time which is long relative to the duration of the excitation pulse, wherein the signals are

detected, the duration of each interval between successive signals is measured, and a relationship relating the interval between the excitation pulse and the emission of each signal to the interval between each signal and the preceding signal in the series is derived to represent the characteristic response.

Preferably the interval between the excitation pulse and the emission of each signal is plotted against the interval between each signal and the preceding signal in the series, a curve is fitted to the plot, the position of the minimum value of the interval between the excitation pulse and the emission of each signal as represented by the curve is determined, and the interval between successive signals corresponding to the position of the minimum is determined to provide a measure of the characteristic response of the medium.

The present invention makes it possible to measure for example a fluorescent decay lifetime from pulse (quantum) signals resulting from only a single excitation pulse of very short duration, for example a few nanoseconds, or less.

The invention is particularly applicable to the analysis of signals resulting from excitation of fluorophores. The signals may result from direct excitation or energy transfer to one species from another species excited by the excitation pulse.

The timing of the signals may be determinined from any convenient portion of each signal, for example the leading edge.

The invention may be applied to the assessment of a large number of samples of a particular medium using a single source. Excitation pulses may be delivered to the samples from the single source and received by a single detector. Each of the samples may receive an excitation pulse in turn, signals from each of the samples being detected in turn, or alternatively each of the samples may receive an excitation pulse simultaneously, with signals from all of the samples being detected in parallel. This latter approach enables rapid assessment of a large number of samples to determine whether any of them is generating a characteristic response before the application of the first approach to assess individual samples which do exhibit some characteristic response.

An embodiment of the present invention will now be described, by way of example, with reference to the accompanying drawings, in which;

Figure 1 illustrates the timing of the emission of photons by a fluorescent sample after excitation by a short-duration excitation pulse;

Figure 2 is a representation of data derived from the events represented in Figure 1; and

Figure 3 is a schematic representation of an apparatus in accordance with the present invention.

Referring to Figure 1, this illustrates the excitation of and emissions from a sample which has fluorescent properties having a characteristic lifetime. An excitation pulse 1 is used to excite the sample and as a result a series of photons is emitted, each photon being detected to generate a signal pulse. The first three photons emitted after the excitation pulse 1 are indicated in Figure 1 by pulses 2, 3 and 4. The arrow 5 in Figure 1 represents the characteristic lifetime of the excited sample.

Referring to Figure 2, this represents data extracted from the events represented in Figure 1. The horizontal axis corresponds to the time interval which has elapsed since excitation when an emission pulse is detected. The lines corresponding to the signal pulses 2, 3 and 4 in Figure 1 are identified by the same numbers in Figure 2. The vertical axis corresponds to the time interval which has elapsed since the preceding signal pulse in the series. Thus the length of the line 3 in Figure 2 corresponds to the time interval between signal pulses 2 and 3 of Figure 1.

A curve 6 is then fitted to the data represented in Figure 2 and exhibits a pronounced minimum at the position indicated by line 7 on the "time since excitation" axis. The time interval represented by the position of the line 7 corresponds to the characteristic lifetime of the sample.

Given that the photon emission pulse signals represented in Figure 1 relate to the decay of an active species, the resulting pulse train will inevitably "bunch" around the characteristic lifetime of that species. Thus although the pulse emission times represented in Figure 1 may appear to be random for any particular emission pulse,

the underlying trend is clear and may be analysed by the simple plotting and curve fitting techniques described with reference to Figure 2.

In noisy environments, correlation or equivalent signal processing techniques may be applied to the emission signals to increase sensitivity.

The described approach allows very rapid analysis, for example analysis of the characteristic decay time of a fluorophore in under four decay lifetimes, that is in a timescale significantly below 1 microsecond for most fluorophores.

The invention may be applied to mass screening, relying upon multiplexing of the detector and/or emitter as described with reference to Figure 3.

Referring to Figure 3, light from a pulsed excitation source 8 is conditioned in an optical conditioning unit 9 which may incorporate for example filters, polarisors, lenses etc and delivered to a multiplexer 10 which delivers excitation pulses of light to each of a series of optical fibres 11. Each of the optical fibres 11 delivers light to a respective sample holder 12, only one of the sample holders being shown. Each of the sample holders 12 is coupled by a respective optical fibre 13 to a detection demultiplexer 14 the output of which is applied via a conditioning unit 15 to a emission pulse detector 16. A processing and storage unit 17 is used to convert the detected pulse data which is represented in Figure 1 into the representation illustrated with reference to Figure 2.

After each excitation light pulse the light emitted from the illuminated sample is detected and stored. The multiplexers may be switched to deliver light to and receive light from only one sample at a time. Alternatively, light may be delivered to and detected from all of the samples simultaneously. This alternative would allow a first pulse to be sent to each sample in parallel, and if no photon emission signal resulted from any of the samples it would not then be necessary to analyse each of the samples in turn. The process could simply move on to the next array of samples. If however one or more of the individual samples did emit a photon, the system could be switched back to the full multiplex configuration in which an excitation pulse would be delivered to each of the samples in turn. The detection multiplexer would switch to each of the samples in turn to thereby allow assessment of a fresh sample even if the

previously assessed sample might still be emitting photons. Any signals emitted by the previously assessed sample would simply not be detected if the demultiplexer had been switched away from that sample.

For fluorophores with lifetimes of 20 nanoseconds or less, the total measurement time for a plate having wells for 96 samples will be less than 1 microsecond per well giving a measurement time for the whole plate of less than 1 millisecond.

The multiplexers may be switchable between true multiplexing and beam splitting configurations. Alternatively the system could be constructed with diffraction pattern generators, scanning devices, mirror assemblies or shutters for example.

Thus the present invention makes it possible to measure the characteristic lifetime of a fluorescent response or other time-dependent response by means of excitation with a single pulse and subsequent timing of the intervals between resultant emitted signals. Signal analysis may include correlation, or equivalent signal processing in real time or after storage. The invention is particularly applicable to fluorescent decay spectroscopy but may be applied in any other media such as radioactive decay or chemical induced decay.

The decay may not be of the triggered species but of a complex (i.e. excimer) or other species which has had energy transfer from another species that has been excited (i.e. fluorescent energy transfer). Only a portion of the emitted signal may be analysed, the remainder of the signal simply being ignored or used as a trigger signal. A multichannel system may utilise many excitation sources and detectors or only a single excitation source and detector with "channels" being formed by multiplexed fibres, pattern generators or other beam splitting and combining methods.

Generally a single excitation pulse will be used but it may be possible to use multiple excitation pulses with a single sample to measure the "bleaching" of the excited species as a further measurement parameter. The term bleaching is used to indicate a situation in which an excited sample is fully excited by a first excitation

pulse and is not therefore significantly affected by a second excitation pulse received before the excitation caused by the first excitation pulse has decayed.

The invention could be applied to single particle detection where the measurement is on an individual particle passing through a flow cell.

Any available techniques may be used for detection of the emission signal pulses resulted from sample excitation. One possible approach to signal detection is that described in British patent application number 9721847.3. That specification describes a timing circuit for recording the duration of an interval between two events, the timing circuit comprising a source of clock pulses and a counter which accumulates the clock pulses occuring between the events, the clock and counter being arranged such that the accumulated count increases at a rate which reduces with increasing interval duration.

CLAIMS

1. A method for assessing the characteristic response of a medium to an excitation pulse of predetermined duration which causes the medium to emit a series of signals over a period of time which is long relative to the duration of the excitation pulse, wherein the signals are detected, the duration of each interval between successive signals is measured, and a relationship relating the interval between the excitation pulse and the emission of each signal to the interval between each signal and the preceding signal in the series is derived to represent the characteristic response.

2. A method according to claim 1, wherein the interval between the excitation pulse and the emission of each signal is plotted against the interval between each signal and the preceding signal in the series, a curve is fitted to the plot, the position of a minimum value of the interval between the excitation pulse and the emission of each signal as represented by the curve is determined, and the interval between successive signals corresponding to the position of the minimum is determined to provide a measure of the characteristic response of the medium.

3. A method according to claim 1 or 2, wherein the signals result from excitation of fluorophores by the excitation pulse.

4. A method according to claim 1 or 2, wherein the signals result from energy transfer to one species from the another species excited by the excitation pulse.

5. A method according to any preceding claim, wherein the timing of the signals is determined from a predetermined portion of each signal.

6. A method according to any preceding claim, wherein excitation pulses are delivered to a plurality of samples of the medium from a single source, and signals from each sample are received by a single detector.

7. A method according to claim 6, wherein each of the plurality of samples receives an excitation pulse in turn, and signals from each of the samples are detected in turn.

8. A method according to claim 6, wherein each of the plurality of samples receives an excitation pulse simultaneously, and signals from all of the samples are detected in parallel.

9. An apparatus for carrying out a method in accordance with any preceding claim, comprising means for detecting each of the series of signals, means for measuring the duration of each interval between successive signals in the series, means for plotting the interval between the excitation pulses and the emission of each signal against the interval between each signal and the preceding signal in the series, means for fitting a curve to the plot, means for determining the position of the minium value of the interval between the excitation pulse and the emission of each signal as represented by the curve, and means for determining the interval between successive signals corresponding to the position of the minimum to provide a measure of the characteristic response of the medium.

10. A method for assessing the characteristic response of a medium to an excitation pulse of predetermined duration substantially as hereinbefore described with reference to the accompanying drawings.

11. An apparatus for assessing the characteristic response of a medium to an excitation pulse of predetermined duration substantially as hereinbefore described.

ABSTRACT

A method and apparatus for assessing the characteristic response of a medium to an excitation pulse of predetermined duration which causes the medium to emit a series of signals over a period of time which is long relative to the duration of the excitation pulse. The signals are detected and the duration of each interval between successive signals is measured. A relationship relating the interval between the excitation pulse and the emission of each signal to the interval between each signal and the preceding signal in the series is derived to represent the characteristic response. The interval between the excitation pulse and the emission of each signal may be plotted against the interval between each signal and the preceding signal in the series and a curve may be fitted to that plot. The position of a minimum value of the interval between the excitation pulse and the emission of each signal as represented by the curve is then determined, and the interval between successive signals corresponding to the position of the minimum is then determined to provide a measure of the characteristic response of the medium.

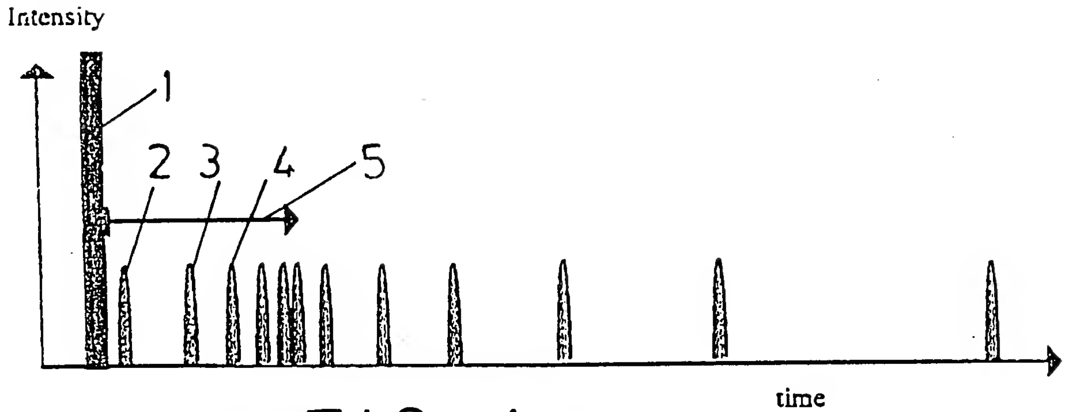

FIG. 1

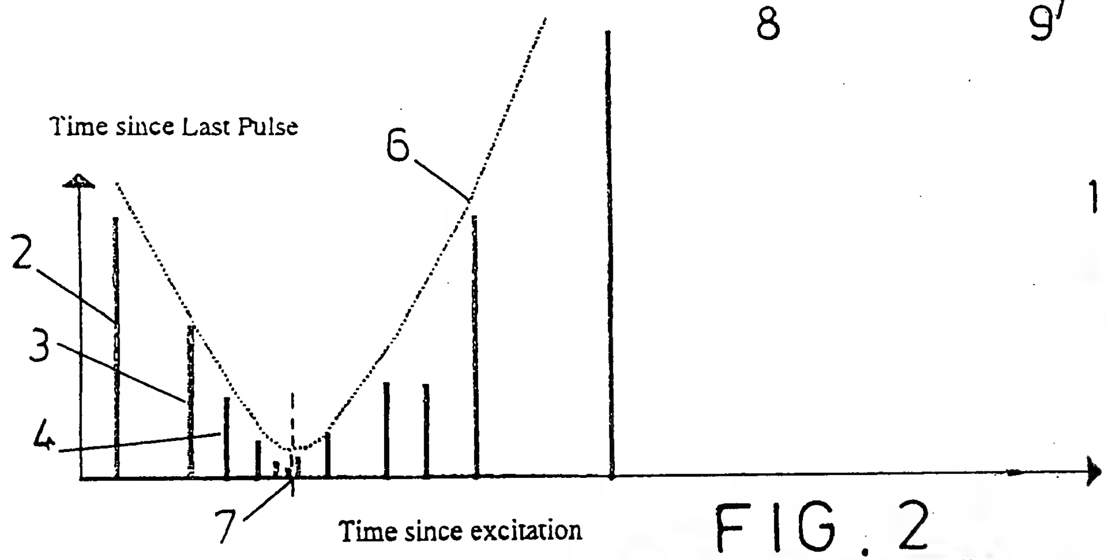

FIG. 2

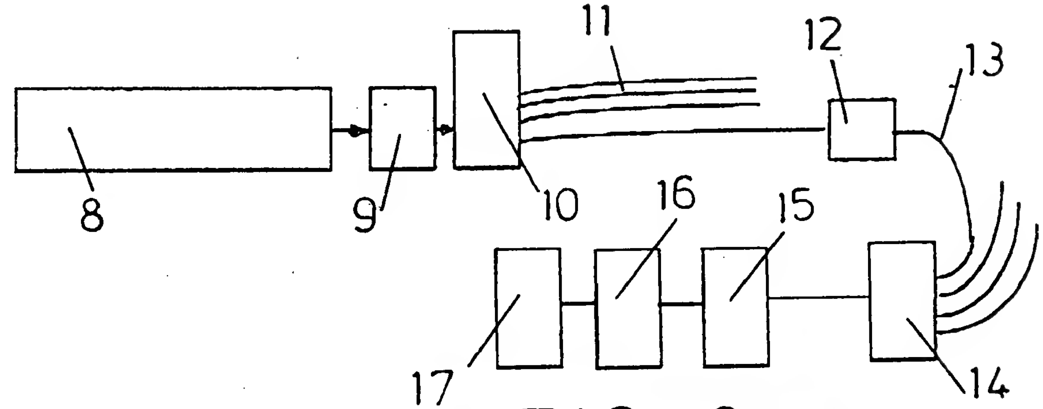

FIG. 3

PCT/GB 99/01989

Marks & Clerk

16.7.99